Research Report

Prepartum vaginal microbiota and postpartum uterine microbiota in cows

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Abstract

Postpartum uterus clears bacteria within 6 weeks; however, up to 40% of dairy cows cannot eliminate uterine infection. Culture-independent approaches based on the analysis of the 16S rRNA gene indentified reduced richness and diversity in the reproductive tract of cows with endometritis. Goal was to characterize the vaginal microbiota before calving and the uterine microbiota after calving in cows that had or not developed endometritis. We compared the results of DNA sequencing with conventional culture methods in uterine samples collected 21 days after calving. Vaginal samples (via cytobrush) from multiparous cows (n = 61) were obtained 1 week before calving (-1w) and uterine samples 1 week (+1w), 3 weeks (+3w), and 5 weeks (+5w) after calving. Samples of healthy cows (n = 11) and cows with endometritis (n = 11) were selected for DNA extraction and 16S rRNA sequencing. Richness and diversity measures in the uterine microbiota were not different between healthy and diseased groups (Chao p = 0.17, Simpson p = 0.29) at -1w, +1w and +5w. Uterine microbiota composition differed (p = 0.03) between groups only at 3 weeks after calving. Diseased cows had a lower relative abundance of *Firmicutes* and *Bacteroidetes* and a higher abundance of *Actinobacteria* than healthy cows. Bacteria that grew in culture were often represented within the most abundant bacterial genera in the DNA sequencing 3 weeks after calving.

Keywords: Microbiota, dairy cows, vagina, uterus

Introduction

Approximately 95% of cows have some degree of uterine contamination with bacteria and inflammation after calving, regardless of signs of disease.[1,2] Loss of anatomical barriers and the negative pressure created uterine contraction and relaxation enhance the vacuum effect and bacterial ascent to the uterus, allowing rapid colonization within minutes.[3,4] There is also evidence that bacteria could reach the uterus through blood circulation.[5] Gram-negative bacteria dominate during the first week after calving, gradually replaced by gram-positive organisms by day 15 postpartum, when 78% of cows still have bacteria in the uterus.[6] During normal uterine involution, the uterus clears bacteria at week 6 after calving;[7] however, up to 40% of dairy cows cannot eliminate infection.[8] Persistence of pathogenic bacteria in the uterus is associated with delayed uterine involution, postpartum uterine diseases (PUDs), and reduced reproductive efficiency.[9-11]

Based on culture-dependent studies, most common bacteria involved in PUDs are *Trueperella pyogenes* (*T. pyogenes*), *Escherichia coli* (*E. coli*), *Fusobacterium necrophorum*, *Prevotella melaninogenica*, and *Bacteroides* spp.[12,13] Culture-independent approaches based on the analysis of 16S rRNA gene (the most conserved ribosomal RNA among bacteria and has the most variability between species) have indentified that dairy cows with PUDs had distinct microbial profiles and reduced richness and diversity compared to healthy cows.[14-18] Nonconventional pathogens have also been identified by DNA sequencing, such as species of *Bacteroides* and *Helcococcus*. In contrast, other bacteria normally associated with diseases, such as *E. coli*, *Mycoplasma* and *Ureaplasma*, were abundant in healthy postpartum cows.[19] It remains unclear whether changes in the normal composition of the uterine microbiota (dysbiosis) are associated with PUDs.[20]

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Most studies investigating PUDs in dairy cows have focused on the early postpartum period before the completion of uterine involution and the reestablishment of a complete partitioning of the reproductive tract.[21] Vaginal microbiota is a potential source of uterine contamination after calving and needs to be investigated and understood.[22]

We hypothesized that prepartum vaginal microbiota and the uterine microbiota in postpartum cows differ between healthy and diseased dairy cows. Objective was to use DNA sequencing to characterize bacterial communities in the genital tract of healthy dairy cows during the peripartum period and make associations with clinical endometritis. We compared the uterine bacteria obtained by conventional culture methods with the microbiota profile of DNA sequencing from samples collected 3 weeks after calving.

Materials and methods

Animals and management

This research was conducted in compliance with the experimental practices and standards approved by the Animal Care Committee of the University of Montréal (#211-03) in compliance with the Canadian Council of Animal Care Guidelines.

Three commercial dairy herds in the same region of Quebec (Canada) were recruited, and multiparous cows (n = 61) were included in the study between June 2016 and February 2017. Reproductive and health data were compiled in a databank using health record management software (DSAHR, Saint-Hyacinthe, Quebec, Canada J2S 3A5). Rolling herd average milk production for the 3 herds was 9,000 kg. All farms had a tied-stall barn system. Cows were milked twice daily and fed a total mixed ration formulated mainly with corn and hay silage to meet the dietary requirements for the various stages of lactation (NRC, 2011).

Farms were visited weekly by the same veterinarians and cows were vaccinated twice (days 40 and 26 before parturition) with 2 ml intramuscular *E. coli* (J-VAC, Merial Inc., Athens, GA, USA) vaccine and once (days 15-40 after calving) with 2 ml intramuscular bovine viral diarrhea virus, types 1 and 2, infectious bovine rhinotracheitis, PI-3, and bovine respiratory syncytial virus (Bovi-Shield GOLD FPTM 5 L5, Zoetis, Parsippany, New Jersey 07054, USA) vaccine, and injected with 5 ml selenium (MU-SE, Intervet Canada Corp., Kirkland, Quebec, Canada H9H 4M7) 60 days before calving.

Sampling and experimental design

Based on the predicted date of calving (DSAHR software), cows were examined 1 week before calving (-1w), then 1 (+1w), 3 (+3w), and 5 weeks (+5w) after calving (Figure 1). Examinations included an assessment of lameness, cyclicity (presence of a corpus luteum) via transrectal and ultrasonographic examinations, body condition, and milk somatic cell count. Blood samples were collected for hematologic profile; visual vaginal discharge[23] and cervical appearance[24] were also performed. Before calving, transrectal examination was performed to confirm pregnancy. It involved the assessment of placentomes, fetus viability, and uterine artery (fremitus).

In addition, a vaginal (fornix) cytobrush sample was collected 1 week before calving and endometrial cytobrush samples were collected at 1, 3, and 5 weeks after calving. After cleaning cow's perineal area with soap and water, 70% isopropyl alcohol was sprayed and dried using paper towels. Visual cervical assessment was via vaginal speculum examination before a sterile cytobrush rod (covered with 2 sterile sanitary sheaths) was introduced into the vagina and guided through the cervix per rectum.[25] Once the tip of the rod reached cervical opening and uterine body, first and second sanitary sheaths were pulled back, respectively, and the cytobrush was pushed out of the rod and rotated against the dorsal wall of the uterine body with gentle pressure of the index finger through the rectum. Cytobrush was retracted into the rod and each sanitary sheath before removed from the cervix and the vagina, respectively. Once outside the genital tract, the cytobrush was gently rolled onto a sterilized microscope slide. The cytobrush was then cut with sterile scissors, placed in a sterile 2 ml cryovial, and stored at –80°C within 5 minutes. For the sampling at +3w, an additional uterine cytobrush sample was collected and transferred into a tube containing an anaerobic transport medium (BBL Culture Swab, BD, Mississauga, ON, Canada), placed on ice and processed in the bacteriology laboratory within 2 hours after collection.

Cytology slides were stained using the May-Grunwald-Giemsa stain, 300 cells (neutrophils and endometrial cells) were counted per slide in multiple fields by one person and the number of polymorphonuclear neutrophils (PMNs) was assessed. Cows were not enrolled in a systematic synchronization protocol, and reproductive data from cows were collected for at least 300 days after calving.

Case definition

No antibiotic was used before and during the sampling period. From the 61 cows, diseased cows (n = 11) were identified

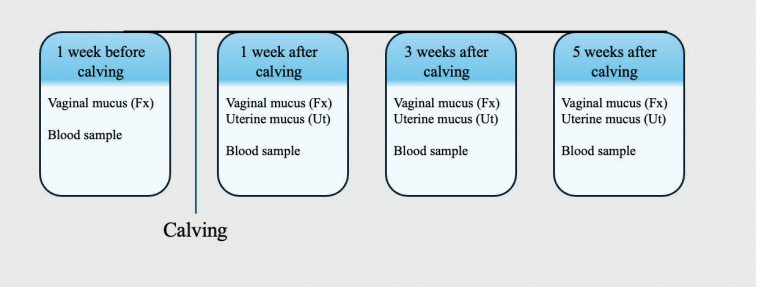

Figure 1. Timeline of sampling and genital examination of prepartum and postpartum dairy cows (n = 22) from 3 dairy herds in Quebec (Fx; fornix, Ut; Uterine mucus)

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based on 2 criteria at 5 weeks after calving: 1. positive clinical endometritis; cows with purulent vaginal discharge (PVD) of grade ≥ 2[23] and 2. positive subclinical endometritis; cows with > 5% polymorphonuclear neutrophils (PMNs) on uterine endometrial cytology (number of PMNs/number of total cells.[26] Control animals (n = 11) were characterized by the absence of all criteria (PVD < 2 and PMNs < 5%).

DNA extraction, 16S rRNA gene sequencing

Cytobrushes were stored at –80 °C after collection until DNA extraction. Frozen samples were dipped in 1 ml of phosphate-buffered saline (PBS) and DNA was extracted from the suspension using the PowerSoil DNA extraction kit (Qiagen) according to the manufacturer's protocol. The V4 region of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the forward 515F (GTGCCAGCMGCCGCGGTAA) and reverse 806R (GGACTACHVGGGTWTCTAAT) primers and the following conditions: denaturing for 3 minutes at 94 °C, followed by 35 cycles of 45 seconds at 94 °C, 60 seconds at 50 °C, and 90 seconds at 72 °C, with a final elongation step of 72 °C for 10 minutes. The PCR products were sequenced in an Illumina MiSeq platform using reagents for 2 x 250 cycles at the McGill University and Génome Québec Innovation Centre.

The mothur software was used to perform the bioinformatic analysis.[27] Contigs assembly was made from the original FASTQ files, excluding sequences longer than 300 bp, containing ambiguities, and having polymers longer than 8 bp. Sequences were aligned using the SILVA 16S rRNA reference database and clustered at 97% similarity. Taxonomic classification was obtained using the Ribosomal Database Project. Richness (total number of species) was estimated by the Chao1 index, and diversity was measured by the Simpson index (number of species accounting for their evenness). For beta diversity, the Bray-Curtis dissimilarity index was used to compare community composition among samples.

Uterine bacterial culture and identification

Uterine cytobrush samples were collected at +3w for routine bacterial culture (aerobic and anaerobic) using standard methods for bacteriological testing (API system, bioMérieux, Marcy l'Etoile, France). Cytobrush samples were stored in a culture tube (BBL Culture Swab, BD, Mississauga, ON, Canada), placed on ice, and transported to the diagnostic laboratory of the Faculty of Veterinary Medicine within 2 hours. For microbiological analysis, the solution was plated onto sheep blood agar with a sterile disposable plastic eye (soy agar with 5% sheep blood, Becton, Dickinson and Co., Sparks, MD, USA). Plates were incubated for 48 hours at 35 °C under aerobic conditions and then examined. When growth was observed, the number of colonies was graded as rare, 0 (1 colony), few, +1 (between 2-5 colonies), +2 (> 5 colonies), and +3 (> 5 colonies after dilution (1 in 2 dilution) whereas colony types were identified based on morphology, pigmentation, and hemolytic patterns.

Beta hemolytic, catalase-negative minuscule colonies demonstrating coliform gram-positive rods were identified as *T. pyogenes*. They were isolated using the standard procedure used at the diagnostic laboratory of the Faculty of Veterinary Medicine (PON-BAC-019). The samples were plated on brucella agar containing neomycin (100 mg/ml) and incubated anaerobically at 35 °C for 5 days for culture of other bacterial species. When gram-negative rods were observed, colonies were examined using the API 20 A gallery system to identify *Fusobacterium necrophorum* and *Prevotella melaninogenica*. For isolation of *E. coli*, cytobrush samples were plated on blood agar and MacConkey agar (Oxoid Inc., Ottawa, ON, Canada) at 37 °C. At the reference laboratory for *E. coli* (EcL; Faculty of Veterinary Medicine, University of Montréal), 5 typical lactose-positive *E. coli* colonies from the MacConkey agar plates were streaked with blood agar for isolation and further identification. Isolates were submitted to 3 biochemical tests (indole spot, Simon's citrate and motility) for confirmation of *E. coli*. Isolates of *E. coli* was stored in tryptic soy broth containing 30% glycerol at –80 °C (Becton, Dickinson and Co., Sparks, Maryland, USA).

Collection and analysis of blood

Blood samples were collected aseptically by coccygeal venipuncture from 3 cows into collection tubes containing EDTA for hematology, including red blood cell count, hemoglobin concentration, packed cell volume, erythrocyte sedimentation rate, corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocytic count, and differential leukocytic count.

Data analyses

Downstream analyses were carried out in RStudio (version 3.6.3; R Core Team, Vienna, Austria) using the ggplot2, vegan, FactoMineR, dplyr, tidyverse, and phyloseq packages unless otherwise stated. Uterine and vaginal alpha diversity metrics were examined for normal distribution by Proc Univariate in RStudio. Repeated measures analysis of variance was performed on all alpha diversity indices, with fixed effects of the day, status, and their interaction with the cows. Differences in uterine bacterial composition were investigated according to disease presence and days after calving, and their outcomes were evaluated using a nonparametric multivariate analysis of variance (PERMANOVA) accounting for repeated measures. Linear discriminant analysis effect size (LEfSe) was used to describe the statistical significance and biological relevance among healthy and diseased cows at the genus level. The LEfSe was performed using the online Galaxy interface with uterine health status as the main class, DIM at sampling as the subclass, and the cow as the subject, using an alpha of 0.05 and an effect size threshold of 3.5. Significance level was set for all statistical tests as p value < 0.05.[28]

Results

Descriptive statistics

The study population consisted of 85 cows, but an initial exclusion resulted in the removal of cows due to culling (n = 5), use of antimicrobials (n = 4), metabolic diseases (n = 5), and missing data (n = 10). A total of 61 cows ended the follow-up period without systemic illness (no dystocia, fetal membranes retention, and mastitis). DNA sequencing of vaginal (prepartum) and uterine (postpartum) samples from 22 cows classified as healthy (n = 11; parity 1.7 ± 0.5 [mean ± SD], and BCS 2.8 ± 0.2) and diseased cows (n = 11; parity 1.9 ± 0.5 [mean ± SD], and BCS 2.7 ± 0.3) was performed. One healthy (9%) and 5 diseased (45%) cows had cervicitis Grade 2 at 5 weeks after calving and no significant hematological changes and somatic cell counts and cyclicity among cows were measured.

Microbial community analyses

A total of 9,480,588 paired-end reads were obtained, of which, 7,820,890 passed all quality control after bioinformatic analysis. Results from the alpha diversity of microbiota of healthy and diseased cows from 1 week before calving to 5 weeks after calving are provided (Figure 2). Overall and at each sampling time, alpha diversity of the uterine microbiota was not different (Chao's p value = 0.17, Simpson's p value = 0.29) between healthy and diseased groups.

The principal coordinate analysis addressing the microbial composition (beta diversity) revealed distinct microbiota profiles (p = 0.03) between healthy and diseased groups at 3 weeks after calving (Figure 3). No difference (p = 0.35) was observed for the other sampling times.

Taxonomic composition

The relative abundance of the most abundant phyla (Figure 4A) and genera (Figure 4B) are illustrated. According to taxonomical annotation, the most common bacterial phyla in the uterine microbiota in both groups of cows were *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Proteobacteria*, and *Tenericutes* (Figure 4). During the postpartum period, cows with endometritis had a lesser relative abundance of *Firmicutes*

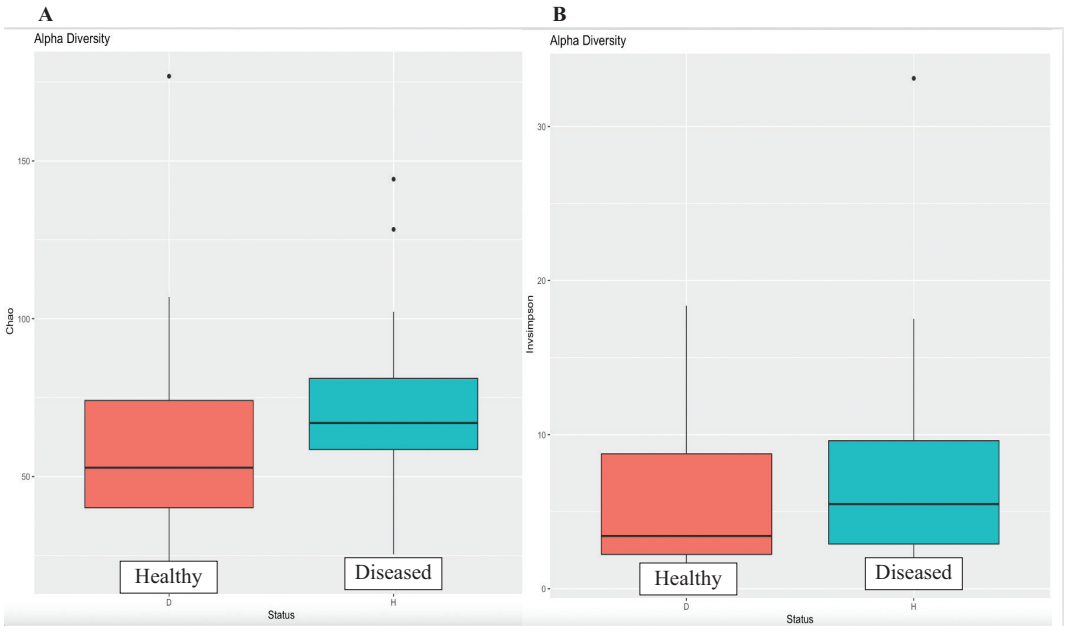

Figure 2. Box plot of the distribution of Alpha diversity indices of healthy and diseased cows from 1 week before calving to 5 weeks after calving. Chao richness estimator (A) and the Simpson diversity index (B) of the uterine microbiota postpartum (n = 22). Healthy (red, n = 11) and diseased (blue, n = 11) cows were identified retrospectively on the vaginal discharge and the number of neutrophils on endometrial cytobrush. The bar inside the box marks the median. Alpha diversity for bacterial genera and phyla were similar between healthy and diseased cows. A repeated measures analysis was performed on all Alpha diversity, with fixed effects of the day, status, and their interaction with the cows. Bars represent SD ($p \leq 0.05$).

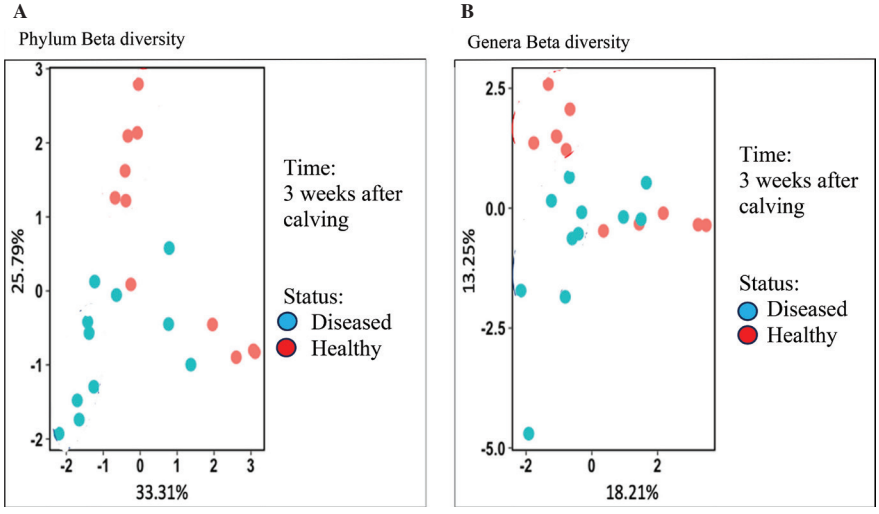

Figure 3. Beta diversity (principal coordinate analysis (Bray–Curtis) for bacteria phyla (A) and genera (B) at three weeks after calving

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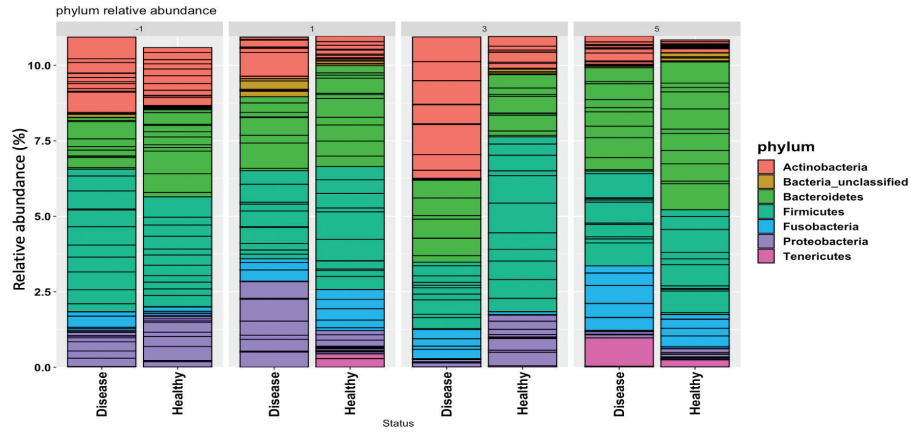

Figure 4A. Color-coded bar plot showing the relative abundance of predominant bacteria at the phylum levels. Relative abundance of the most dominant bacterial phyla in dairy cows (n = 22) in samples collected 1 week before calving and 1 week, 3 weeks, and5 weeks after calving. Only the seven most common phyla are represented (98% of the reads). Based on their uterine health status in 5th week postpartum, cows were retrospectively selected and classified as healthy and diseased groups.

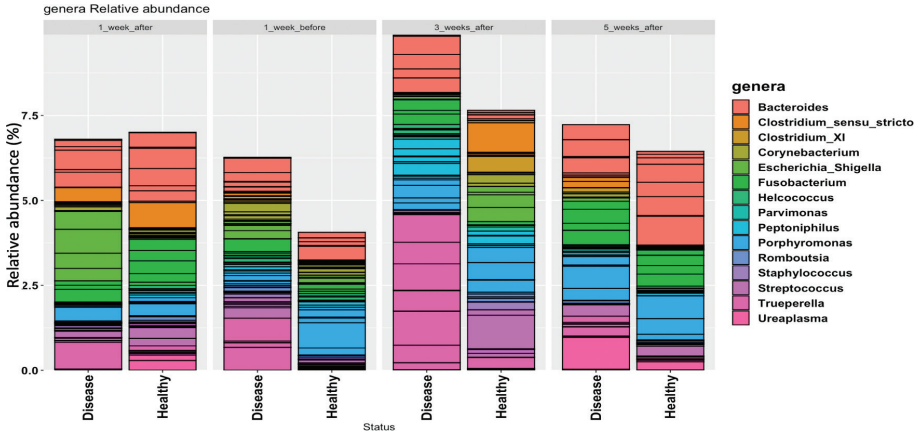

Figure 4B. Relative abundance of predominant bacteria at the genus level. Samples were collected one week before and one, three and five weeks after calving. Only the 14 most common genera are represented. Based on their uterine health status in the 5th week postpartum, cows were retrospectively selected and classified as healthy and diseased groups.

and *Bacteroidetes* and a higher abundance of *Actinobacteria* than healthy cows. The number of unclassified bacteria was < 2% in all samplings.

As per taxonomic assignment the dominant uterine bacterial genera were *Bacteroides, Trueperella, Porphyromonas, Fusobacterium, Escherichia, Streptococcus,* and *Clostridium sensu stricto* (Figure 5). At the genus level, diseased cows had a higher relative abundance of *Bacteroides, Trueperella, Fusobacterium,* and *Peptoniphilus* than healthy cows at 3 weeks after calving. Diseased cows also had a higher relative abundance (p = 0.03) of *Trueperella* from 1 week before to 5 weeks after calving than healthy cows (Figure 6).

Linear discriminant analysis effect size

Diseased cows had discriminately higher abundances (LDA sores > 3.6) of *Helcococcus*, *Peptoniphilus,* and *Trueperella* than healthy cows at 3 weeks after calving (Figure 6). No significant differences between healthy and diseased cows were observed at the other samplings.

Bacterial culture

Gram-positive and gram-negative bacteria were cultured from the cytobrushes of healthy and diseased cows 3 weeks after calving. Most of the diseased cows had *T. pyogenes* in large amounts (9 out of 11, more than 5 colonies) and small amounts of *E. coli* (3 out of 11, 1 colony), *Enterococcus* (3 out of 11, 1 colony), and *Streptococcus uberis* (2 out of 11, 1 colony). Among healthy cows, only 4 out of 11 had a few colonies of *Staphylococcus* spp. (1 colony), *Corynebacterium* (1colony), and *Bacillus* spp. (1 colony).

Discussion

Objective of this study was to use DNA sequencing to investigate bacterial dynamics in prepartum vagina and in postpartum uterus of cows that remained healthy with those that developed endometritis during the transition period. Our results revealed that cows developing endometritis had distinct profiles of the uterine microbiota 3 weeks after calving compared to cows that remained healthy. This suggested that

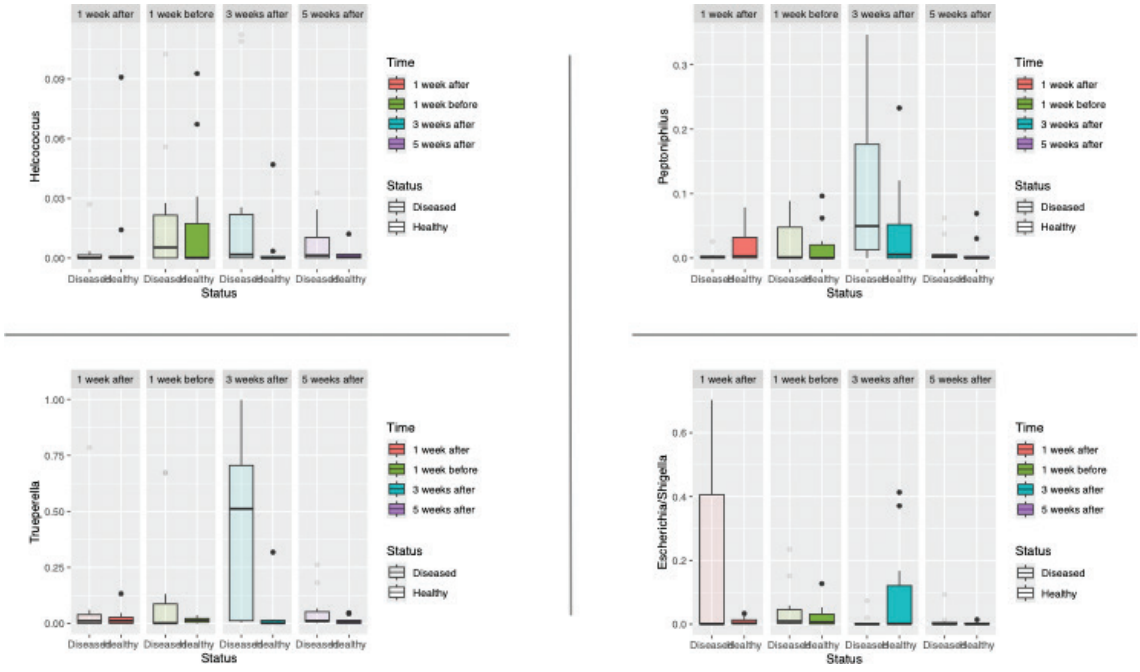

Figure 5. Relative abundance of the most dominant bacterial genera in healthy and diseased postpartum dairy cows (n = 22) in samples collected 1 week before, and 1 week after, 3 weeks, and 5 weeks after calving. Cows were retrospectively selected based on their uterine health status in the 5th week postpartum and classified as healthy (n = 11), and diseased cows with clinical and subclinical endometritis (n = 11; > 50% purulent vaginal discharge and > 5% endometrial PMN). No differences (p > 0.20) in relative abundance in bacteria genera between healthy and diseased cows.

the uterine microbiota goes through early changes before clinical signs of the disease that could be used to predict cows at higher risk by using key bacterial markers.

Our data also confirmed that known uterine pathogens are associated with clinical endometritis. Similar results were observed[29] in the phylum and genera levels in cows with clinical and subclinical endometritis compared to healthy cows in equivalent times (days 10, 21, and 35 postpartum). In the present study, *T. pyogenes* was the most abundant bacteria in 9 of the 11 diseased cows by culture-dependent technique. Other studies observed that *T. pyogenes* was present in the uterus of most postpartum cows with endometritis using culture-independent methods[30] and culture-dependent methods.[31] Our results from sequencing of the 16S rRNA gene supported that cows with endometritis had an increased relative abundance of *Bacteroidetes* and *Actinobacteria* at the phylum level, as well as *Bacteroides* and *Trueperella* spp. at the genus level.

Interestingly, bacteria that grew in culture often appeared within the most abundant bacteria in the16S rRNA gene sequencing. The culture-dependent results have identified several genera (*E. coli*, *Enterococcus*, *Streptococcus uberis*, *Staphylococcus spp.*, *Corynebacterium*, and *Bacillus spp.*) in a very small number (1 colony). These bacteria are among the most common isolated intrauterine bacteria and have been described as potential or opportunistic pathogens[18,32,33]

Streptococci and *Staphylococci* were isolated in postpartum cows with a reduced risk of endometritis.[12] Postpartum dairy cows with intrauterine *a*-hemolytic streptococci had improved reproductive performance.[34] These bacteria may interact with recognized pathogenic members of the uterine microbiota to favor a healthy or diseased uterine cavity. In the present study, *Romboutsia* and other bacteria unclassified at the genus level were associated with a healthy uterine environment, suggesting that further studies searching for the markers capable of predicting the occurrence of diseases are justified.

In addition, *Truperella* spp., *Peptoniphilus* and *Helcococcus* were significantly higher (LDA sores > 3.5) in the disease group compared to the healthy group at 3 weeks after calving (Figure 6). Intrauterine infusion of *T. pyogenes* in Holstein heifers has caused clinical endometritis.[35] Many factors influence bacterial pathogenicity, including bacterial load, the presence of virulence factors, and positive interactions among species.[36] In fact, *T. pyogenes* has a synergic interaction with *F. necrophorum* and *P. melaninogenica* to support their growth and colonization of the uterine cavity around 3 weeks postpartum.[36] As *Helcococus* and *Peptoniphilis* belong to the *Fusobacterium* cooccurrence group, they may act synergistically with *T. pyogenes* and facilitate the uterine dysbiosis observed 3 weeks after calving and increase the risk of clinical endometritis. This synergic interaction between *Trueperella* and the gram-negative anaerobes like *Fusobacterium* has been suggested as a mechanism to overcome uterine defence, culminating with endometritis.[9,33,36,37] A strong co-occurrence between obligate anaerobes like *Peptoniphilus spp*. in postpartum and more specifically, around 35 days after calving was also observed.[29] In addition to the bacterial load and virulence factors, the sequence at which bacteria grow in the uterus may affect the risk of PUDs. However, there was no association between *T. pyogenes* and *F. necrophorum* in cows with clinical endometritis at 5 weeks after calving[38]; therefore, larger studies are needed.

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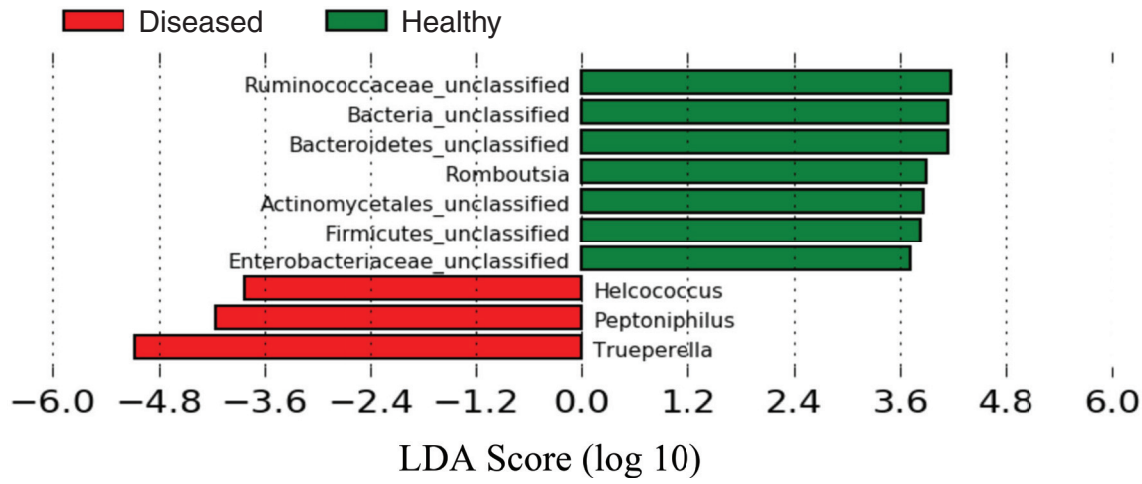

Figure 6. Linear discriminant analysis (LDA) effect size plot showing the differences in uterine microbiota among cows diagnosed in the 5 weeks after calving as healthy (n = 11), and diseased cows (n = 11; > 50% purulent vaginal discharge and > 5% endometrial PMN). Histograms show the LDA effect size computed for features at the genera levels. Enriched features for healthy cows are indicated with positive LDA scores (green), and enriched features in diseased cows are indicated with negative LDA scores (red). Only features with p > 0.05 and an effect size cut-off of 3.5 are plotted.

E. coli is detected in the uterus within a few days of calving before the invasion of other bacteria and is more associated with metritis in the early postpartum than endometritis. Using culture-dependent and PCR-based methods, *E. coli* was isolated between 1 and 3 days after calving.[7,14] In the present study, healthy cows had a higher relative abundance of *Escherichia/Shigella* than diseased cows 1 week after calving. Likewise, rare *E. coli* in the uterus of dairy cows with metritis was identified by metagenomic sequencing analysis.[4] In other studies, *E. coli* was identified more frequently in cows with metritis than in healthy cows.[38] The lack of knowledge on the interaction between *E. coli* and other bacteria like *T. pyogenes* and *F. necrophorum* and the diversity of *E. coli* strains (virulent factors) may explain the conflicting results. In the present study, virulent factors of *E. coli* were not assessed.

Based on the rationale that the ascending colonization of the uterus from the vagina is the most evident pathway for PUDs, the investigation of the vaginal microbiota before calving may improve our understanding of the sequential events leading to clinical endometritis. *Frimicutes, Bacteroidetes, Proteobacteria,* and *Actinobacteria* were isoalated in the vagina of cows during the postpartum period.[39–42] In the present study, the dominant bacterial genera in the vagina were *Bacteroides, Trueperella, Porphyromonas, Fusobacterium, Escherichia/Shigella, Streptococcus, and Clostridium sensu stricto*. Vaginal microbiota in dairy cows was associated with the occurrence of reproductive tract diseases.[43] However, an important point of the present results is that *Trueperella* and *Fusobacterium* were present in the vagina 1 week before calving, sustaining the importance of these 2 species in the ascending colonization of the uterus after calving and their role in the etiology of endometritis in dairy cows in the postpartum period. These results are in line with the study[14] that identified *Fusobacteria* in the vagina of dairy cows 7 days before calving.

Genital microbiome may vary among species and even individuals of the same species,[4] with nutrition, farm management[19] and phase of estrous cycle[44] influencing the microbiota composition.[45,46] Nevertheless, breeds under various management can present similarities in their vaginal microbiota[41] and no difference between sampling techniques or phase of estrous cyle were observed.[47] The 3 farms of the present study were localized close to each other and had similar herd health and feeding management reducing data variation since the geographic origin may be a significant factor of variation of the microbiome.[48] All animals were of the same breed (Holstein) and had similar body conditions at sampling time. Limitations of the present study included small sample size, short sampling interval before calving and short interval of sampling (9 months) during the year. Nevertheless, *Trueperella* spp. in the vagina of cows before calving, with its increased relative abundance in the uterus at 3 weeks after calving, suggests that *Trueperella* could be used as a predictor of postpartum clinical endometritis in dairy cows and could justify larger prospective studies to test the ability of this marker to predict endometritis in a broader population and eventually be used as an accurate and rapid test for prognosis of PUDs.

Conclusion

The microbiota composition of the uterine microbiome was significantly different between healthy and diseased cows before clinical signs of endometritis (3 weeks postpartum). In cows with clinical endometritis, *T. pyogenes*, *Peptoniphilus,* and *Helcococcus* were discriminately in higher abundance than in healthy cows. *T. pyogenes* in the vagina of cows before calving and its increased relative abundance in the uterus 3 weeks after calving suggested that bacterial markers before calving could also be used as predictors of postpartum clinical endometritis in dairy cows. Larger prospective studies to test the ability of these markers to predict endometritis should be performed.

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Conflict of interest

None to report.

Authors' contribution

AB collected data, analysed and interpreted data, and drafted the mansucript; MC analysed and interpretated data; MS reviewed; RL conceptualized and designed the study, and refined the manuscript; and all authors approved submission.

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